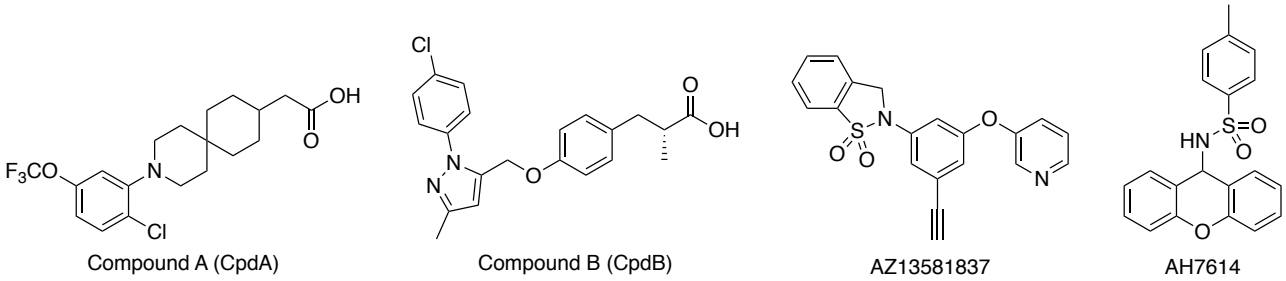

Figure S1. Chemical structures of FFAR4 selective ligands. Chemical structures of the FFAR4 selective agonists A) CpdA [23], B) Cpd B (Metabolex-36) [21], and C) AZ13581837 [26]. D) Chemical structure of the FFAR4 selective antagonist AH7614 [27].

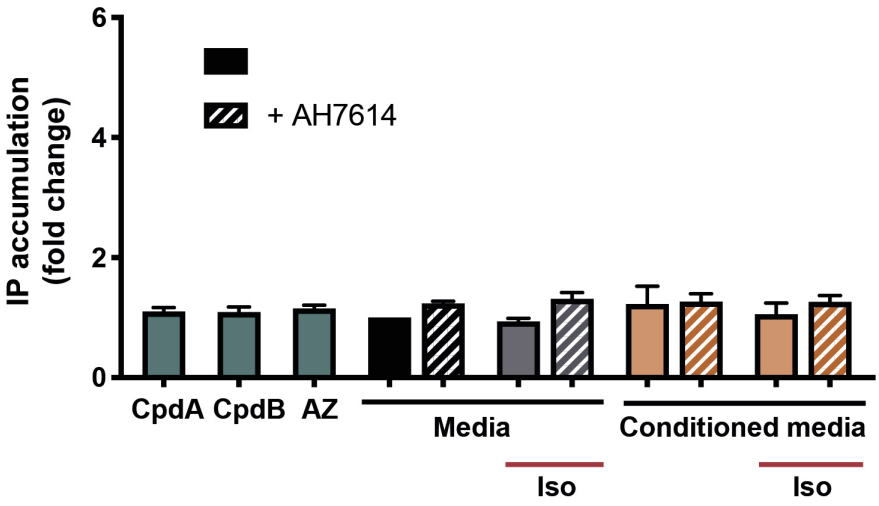

Figure S2. IP accumulation assay control cells. IP accumulation in COS-7 cells transiently transfected with the empty vector PCMV-Tag2b (n=5). Cells were stimulated with conditioned media from murine adipocytes with or without stimulation of isoproterenol and with or without the specific, synthetic FFAR4 antagonist AH7614. The selective, synthetic FFAR4 agonists CpdA (1μM), CpdB (1μM) and AZ (1μM) were used as positive controls.

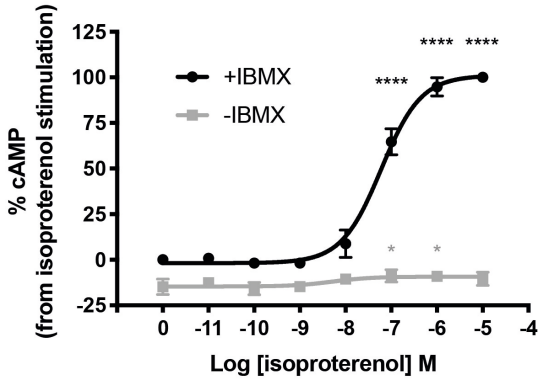

Figure S3. cAMP in murine adipocytes. Isoproterenol stimulated cAMP release in murine adipocytes in the absence (black curve) and presence of IBMX (grey curve) (n=7). Data are presented as SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 using Dunnet's multiple comparison test.

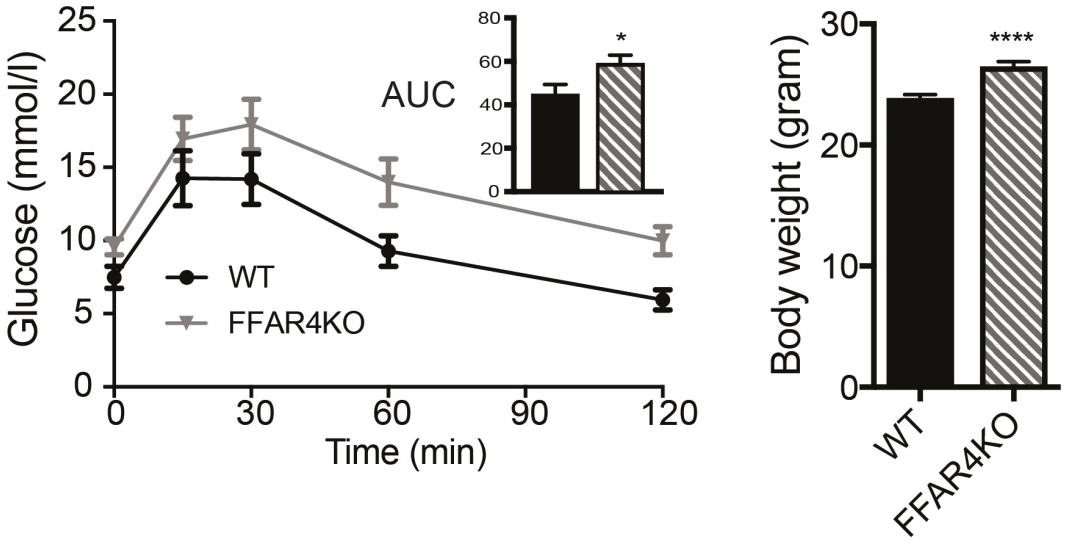

Figure S4. Glucose tolerance test in FFAR4KO mice. A) OGTT in WT and FFAR4KO mice fasted for 6 hours prior to the study (n=8). B) Body weight of the WT and FFAR4 mice at 12 weeks of age (n=30). Data are presented as SEM. *p<0.05, **p<0.01, ***p<0.001, using students t-test.

row m/z	RT	ID
311.296	7.915201467	Arachidic acid
303.233	7.557570406	Arachidonic acid
339.327	8.071604935	Behenic acid
327.233	7.544981866	Docosahexaenoic acid
199.171	7.120782301	Dodecanoic acid
337.311	7.928218906	Erucic acid
325.311	7.964932873	Heneicosanoic acid
269.248	7.713594143	Heptadecanoic acid
367.359	8.314496743	Lignoceric acid
279.234	7.575430748	Linoleic acid
277.217	7.449963409	Linolenic acid
227.202	7.442427825	Myristic acid
365.342	8.07504748	Nervonic acid
297.278	7.849912402	Nonadecanoic acid
281.248	7.67865575	Oleic acid
255.233	7.643677231	Palmitic acid
253.218	7.512929961	Palmitoleic acid
241.218	7.546879169	Pentadecanoic acid
283.264	7.783323987	Stearic acid
353.342	8.183772506	Tricosanoic acid
213.186	7.247322405	Tridecanoic acid
185.155	6.967276573	Undecanoic acid

Table S1. Table of LCFAs analyzed in LC-MS and text for setup. A sample volume of 5 ll was injected into the mobile phase A consisting of 0.1 % formic acid (Sigma-Aldrich, Germany) in milli-Q water with sequential linear concentration gradients to mobile phase B consisting of 0.1 % formic acid in 70:30 (v/v) ACN:MeOH over 7 min with a 1 min post-time: start condition (0 % B), 1 min (8 % B), 2 min (15 % B), 3 min (40 % B), 4 min (70 % B), 4.5 min (100 % B), 6.4 min (100 % B), 6.6 min (5 % B), 7 min (5 % B). A simultaneous set of linear flow gradients were injected as follows: start condition 0.5 mL/min, 2 min 0.6 mL/min, 3 min 0.7 mL/min, 4 min 0.8 mL/min, 4.5 min 1 mL/min, 5 min 1.2 mL/min, 6.4 min 1.2 mL/min, 6.6 min 1 mL/min, 6.8 min 0.5 mL/min, 7 min 0.5 mL/min.

	Mouse (lean)			Human (obese)								
	epididymal adipose tissue			subcutaneous adipose tissue			mesenteric adipose tissue			omental adipose tissue		
	vehicle	isoproterenol	p-value	vehicle	isoproterenol	p-value	vehicle	isoproterenol	p-value	vehicle	isoproterenol	p-value
Arachidic acid	56.34(8.02)	92.01(16.31)	0.0068 (**)	52.77(7.15)	50.38(16.30)	0.7644 (ns)	43.48(10.51)	54.60(18.46)	0.2134 (ns)	53.92(3.50)	53.59(18.46)	0.9654 (ns)
Arachidonic acid	175.31(72.43)	1049.18(357.24)	0.0003(***)	20.55(11.12)	42.97(14.81)	0.0279 (*)	21.96(14.00)	35.45(15.14)	0.0533 (ns)	21.09(12.71)	41.54(15.14)	0.1086 (ns)
Behenic acid	18.57(11.58)	22.94(8.79)	0.4974 (ns)	14.25(9.15)	10.85(3.63)	0.3694 (ns)	9.47(1.96)	11.13(6.11)	0.5657 (ns)	12.11(2.54)	9.37(6.11)	0.3768 (ns)
Docosahexaenoic acid	87.06(49.49)	446.08(104.39)	0.0001 (***)	2.54(2.05)	4.77(4.51)	0.1822 (ns)	2.81(2.80)	8.47(7.59)	0.0544 (ns)	2.43(2.39)	6.22(7.59)	0.0922 (ns)
Dodecanoic acid	26.89(7.82)	65.54(25.14)	0.0117 (*)	6.86(2.22)	5.73(3.68)	0.2564 (ns)	5.59(2.85)	5.23(2.98)	0.8717 (ns)	5.55(3.45)	8.06(2.98)	0.0857 (ns)
Erucic acid	3.90(2.17)	20.21(9.12)	0.005 (**)	1.90(1.00)	2.65(2.04)	0.5326 (ns)	3.54(2.00)	4.70(2.33)	0.2402 (ns)	1.59(1.08)	2.40(2.33)	0.4986 (ns)
Heneicosanoic acid	2.97(4.21)	4.33(2.83)	0.5593 (ns)	2.55(1.16)	1.71(0.68)	0.1607 (ns)	1.78(0.68)	3.12(2.99)	0.3598 (ns)	2.27(0.91)	0.93(2.99)	0.0488 (*)
Heptadecanoic acid	91.55(12.27)	279.64(50.58)	0.0001 (***)	53.64(24.95)	64.73(23.42)	0.4674 (ns)	50.88(6.45)	64.19(46.03)	0.5209 (ns)	63.30(39.10)	57.21(46.03)	0.6602 (ns)
Lignoceric acid	71.91(141.55)	44.50(47.00)	0.6325 (ns)	43.08(44.08)	25.87(13.52)	0.4573 (ns)	12.93(4.78)	20.34(14.16)	0.2802 (ns)	16.60(4.45)	17.13(14.16)	0.9266 (ns)
Linoleic acid	1603.33(1211.61)	36053.92(9066.78)	<0.0001 (****)	143.28(115.06)	299.87(199.08)	0.0443 (*)	93.68(90.00)	306.13(190.07)	0.0071 (**)	101.06(61.77)	299.17(190.07)	0.0979 (ns)
Linolenic acid	112.94(57.36)	2905.79(881.17)	0.0001 (***)	7.96(7.63)	15.25(6.95)	0.0777 (ns)	6.27(6.75)	20.91(12.01)	0.0062 (**)	6.91(3.20)	21.78(12.01)	0.1223 (ns)
Myristic acid	81.47(20.32)	232.83(48.57)	0.0003(***)	30.87(5.57)	35.11(11.45)	0.5014 (ns)	28.77(8.18)	36.45(11.32)	0.0717 (ns)	39.69(18.88)	36.97(11.32)	0.6646 (ns)
Nervonic acid	1.51(2.32)	5.68(6.31)	0.1868 (ns)	1.09(0.73)	0.81(0.46)	0.4947 (ns)	1.17(0.62)	2.89(5.52)	0.4530 (ns)	0.56(0.13)	1.08(5.52)	0.1709 (ns)
Nonadecanoic acid	14.47(4.84)	46.81(14.70)	0.0012 (**)	6.78(2.49)	8.49(5.10)	0.5436 (ns)	7.59(4.18)	8.84(4.86)	0.6886 (ns)	7.66(2.43)	10.36(4.86)	0.5110 (ns)
2Oleic acid	1450.07(546.51)	26029.65(5710.84)	<0.0001 (****)	688.95(390.53)	1113.46(564.15)	0.0643 (ns)	590.55(368.92)	1344.04(396.24)	<0.0001 (****)	630.25(312.60)	1689.38(396.24)	0.0692 (ns)
Palmitic acid	1800.07(279.44)	12301.00(1684.54)	<0.0001 (****)	1158.70(120.20)	1577.31(431.29)	0.0501 (ns)	1156.39(112.28)	1726.01(526.14)	0.0398 (*)	1277.12(259.37)	1559.93(526.14)	0.1255 (ns)
Palmitoleic acid	180.09(86.21)	5055.45(1506.79)	0.0001 (***)	28.58(16.97)	61.53(38.64)	0.0312 (*)	26.55(15.53)	80.92(59.53)	0.0300(*)	27.90(9.05)	71.16(59.53)	0.0008 (***)
Pentadecanoic acid	49.85(9.48)	78.92(38.05)	0.1187 (ns)	15.67(7.53)	17.12(5.32)	0.7347 (ns)	12.44(2.34)	23.55(8.75)	0.0205 (*)	29.89(5.10)	26.77(8.75)	0.1189 (ns)
Stearic acid	2832.60(579.08)	3505.38(995.29)	0.1960 (ns)	2280.63(556.26)	2369.49(590.56)	0.8346 (ns)	2349.30(603.20)	2262.62(371.81)	0.7835 (ns)	2527.53(119.29)	2252.13(371.81)	0.2123 (ns)
Tricosanoic acid	11.17(23.29)	10.47(5.94)	0.9374 (ns)	5.67(7.31)	3.89(1.14)	0.5728 (ns)	4.07(2.93)	3.84(3.20)	0.8989 (ns)	3.35(2.62)	2.69(3.20)	0.6384 (ns)
Tridecanoic acid	3.37(1.98)	8.24(7.45)	0.1172 (ns)	0.85(0.36)	2.06(2.46)	0.2706 (ns)	1.07(0.82)	1.62(0.92)	0.2662 (ns)	0.95(0.80)	2.51(0.92)	0.1199 (ns)
Undecanoic acid	5.79(4.42)	18.88(6.99)	0.0182 (*)	1.53(0.70)	1.57(0.96)	0.9228 (ns)	2.61(2.65)	2.44(1.37)	0.8705 (ns)	1.30(0.80)	2.70(1.37)	0.2892 (ns)

Table S2. LC-MS data for targeted LCFAs from murine and human adipocytes.
Data are presented as SEM for panel A. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 using a paired t-test.